X-RAY STRUCTURAL STUDIES OF SOME NATURAL PRODUCTS FROM *RAUWENHOFFIA SIAMENSIS* SCHEFF., *PRISMATOMERIS MALAYANA* RIDL., *CRATOXYLUM FORMOSUM* SSP. *PRUNIFOLUM* AND *ANDROGRAPHIS PANICULATA* NEES PLANTS

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2008

ACKNOWLEDGEMENT

First of all, I would like to offer my most grateful thanks to my supervisor, Dr. Abdul Razak Ibrahim and co-supervisor, Professor Fun Hoong Kun for their guidance, valuable advice, encouragement, patience and understanding throughout my research. I am also deeply grateful to my external supervisor, Dr. Suchada Chantrapromma and researchers from Thailand and Universiti Putra Malaysia for their help, useful advice and encouragement.

I would also like to thank the Malaysian Government and Universiti Sains Malaysia for the post of research officer through the Scientific Advancement Grant Allocation (SAGA) grant no. 304/PFIZIK/653003/A118, Science Fund grant No. 305 / PFIZIK / 613312 and the USM short-term grant no. 304/PFIZIK/635028 which give me the opportunity to carry out my research. I am grateful to the School of Physics, Universiti Sains Malaysia for allowing me to use the equipment in the X-ray Crystallography Laboratory for my research. Also, my special thanks to the Institut Pengajian Siswazah (IPS), Universiti Sains Malaysia for the opportunity to further my masters degree.

Special thanks to Encik K. Karunakaran for the support and assistance during my research in the X-ray Crystallography Laboratory. I am also grateful to all of my fellow friends for their moral support.

Finally, I would like to thank all my family members, my father Ng Tiang Nyo, my mother Tan Chin Kim, my brother Ng Heng Leong and my sister Ng Shue Ki for their valuable comments, encouragement and understanding.

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LIST OF ABBREVIATIONS

CCD	Charge-Coupled Device
GooF	Goodness of Fit
ORTEP	Oak Ridge Thermal Ellipsoid Plot
R	Reliability Index
SADABS	Siemens Area Detector Absorption Correction
SAINT	SAX Area-detector Integration (SAX-Siemens Analytical X-ray)
SMART	Siemens Molecular Analysis Research Tools
TLC	Thin Layer Chromatography
wR	Weighted Reliability Index

KAJIAN STRUKTUR SINAR-X BEBERAPA PRODUK SEMULAJADI DARIPADA TUMBUH-TUMBUHAN *RAUWENHOFFIA SIAMENSIS* SCHEFF., *PRISMATOMERIS MALAYANA* RIDL., *CRATOXYLUM FORMOSUM* SSP. *PRUNIFOLUM* AND *ANDROGRAPHIS PANICULATA* NEES

ABSTRAK

Di dalam tesis ini kaedah kristalografi sinar-X hablur tunggal telah digunakan untuk menyelesaikan dan menentukan lima struktur produk semulajadi yang baru. Struktur molekul 1-(2,4-dihydroxy-6-methoxyphenyl)-3-(4-methoxyphenyl)propan-1-one, $C_{17}H_{18}O_5$, yang diasingkan dari *Rauwenhoffia siamensis* Scheff mempunyai dua molekul yang bebas secara kristalografinya dalam unit asimetrik. Sudut dihedral di antara dua gelang benzena adalah 80.81 $(7)^o$ dalam salah satu molekul dan 65.89 $(7)^o$ bagi molekul yang satu lagi. Molekul yang berhubungan secara simetri itu disambung melalui ikatan hidrogen intermolekul O—H---O untuk membentuk rantaian sepanjang arah [201]. Struktur ini terhablur dalam sistem monoklinik dengan kumpulan ruang $P2_1/c$.

Struktur molekul 1-3-dihydroxy-2-methyl-9,10-anthraquinone, $C_{15}H_{10}O_4$, yang diasingkan dari akar *Prismatomeris malayana* Ridl., adalah planar. Ikatan hidrogen intramolekul O—H---O dan C—H---O dapat dilihat dalam struktur molekul. Molekul-molekul tersebut membentuk dimer yang berpusat simetri melalui ikatan hidrogen intermolekul O—H---O. Struktur hablur ini

diperkukuhkan lagi oleh interaksi $\pi - \pi$ yang lemah. Struktur ini terhablur dalam sistem monoklinik dengan kumpulan ruang $P2_1/c$.

Bagi struktur 12-(1,1-dimethyl-2-propenyl)-5,9,10-trihydroxy-2,2-dimethyl-2*H*,6*H*-pyrano[3,2-*b*]xanthen-6-one, $C_{23}H_{22}O_6$, gelang xanthena dalam sistem ini adalah planar dan gelang kromena adalah dalam keadaan konformasi perahu berpintal. Kedudukan bagi bahagian 1,1-dimethyl-2-propenyl adalah sama satah dengan gelang yang tersambung. Terdapat ikatan intramolekul O—H---O dalam struktur. Molekul-molekul tersebut membentuk dimer yang berpusat simetri melalui interaksi intermolekul C—H---O yang lemah. Molekul-molekul bersambungan oleh ikatan hidrogen intermolekul O—H---O untuk membentuk rantaian satu dimensi sepanjang arah [010]. Struktur ini terhablur dalam sistem monoklinik dengan kumpulan ruang $P2_1/c$.

Struktur 3,19-(2-bromobenzylidene)andrographolide, $C_{27}H_{33}BrO_5$, satu terbitan andrografolida, telah disepara sistesis dengan menggunakan andrografolida sebagai bahan permulaan. Struktur ini terdiri daripada cantuman tiga gelang segienam yang mempunyai konformasi kerusi dan gelang segilima yang mempunyai konformasi sampul. Kumpulan 2-bromofenil terkilas keluar dari gelang yang bersambung. Ikatan hidrogen O—H---O dalam struktur membentuk rantaian sepanjang paksi *b* yang berhubungan antara satu sama lain melalui interaksi C—H---O. Struktur ini terhablur dalam sistem ortorombik dengan kumpulan ruang $P2_12_12_1$.

Struktur 3,19-(2,6-dimethoxybenzylidene)andrographolide, $C_{29}H_{38}O_7$, satu analog andrografolida, telah disepara sistesis daripada andrografolida. Struktur ini terdiri daripada cantuman tiga gelang segienam yang mempunyai konformasi kerusi dan gelang segilima yang mempunyai konformasi berpintal. Ikatan hidrogen O—H---O dalam struktur membentuk rantaian sepanjang paksi *a* yang berhubungan antara satu sama lain melalui interaksi C—H---π. Struktur ini terhablur dalam sistem ortorombik dengan kumpulan ruang $P2_12_12_1$.

X-RAY STRUCTURAL STUDIES OF SOME NATURAL PRODUCTS FROM *RAUWENHOFFIA SIAMENSIS* SCHEFF., *PRISMATOMERIS MALAYANA* RIDL., *CRATOXYLUM FORMOSUM* SSP. *PRUNIFOLUM* AND *ANDROGRAPHIS PANICULATA* NEES PLANTS

ABSTRACT

In this thesis five new structures in the natural product compounds have been solved by single crystal X-ray crystallography method. The structure 1-(2,4-dihydroxy-6-methoxyphenyl)-3-(4-methoxyphenyl)propan-1-one, $C_{17}H_{18}O_5$, which was isolated from the leaves of *Rauwenhoffia siamensis* Scheff has two crystallographically independent molecules in the asymmetric unit. The dihedral angle between the two benzene rings is 80.81 (7)^o in one molecule and 65.89 (7) ^{o} in the other. The symmetry related molecules are linked via O—H---O intermolecular hydrogen bonds to form chains along [201] direction. This structure crystallized in the monoclinic system with the space group $P_{2_1/c}$.

The structure of 1-3-dihydroxy-2-methyl-9,10-anthraquinone, $C_{15}H_{10}O_4$, which was isolated from the roots of *Prismatomeris malayana* Ridl., are coplanar. Intramolecular O—H---O and C—H---O hydrogen bonds can be observed in the molecular structure. The molecules form centrosymmetric hydrogen-bonded dimers *via* intermolecular O—H---O hydrogen bonds. The crystal structure is further stabilized by weak $\pi - \pi$ interactions. This structure crystallized in the monoclinic system with the space group $P_{2_1/c}$.

For the structure of 12-(1,1-dimethyl-2-propenyl)-5,9,10-trihydroxy-2,2-dimethyl-2*H*,6*H*-pyrano[3,2-*b*]xanthen-6-one, $C_{23}H_{22}O_6$, the xanthene ring system is essentially planar and the chromene ring is in a screw-boat conformation. The 1,1-dimethyl-2-propenyl substituent is coplanar with the attached ring. O—H---O intramolecular hydrogen bonds are observed in the structure. The molecules form centrosymmetric hydrogen-bonded dimers *via* weak intermolecular C—H---O interactions. The molecules are linked by intermolecular O—H---O hydrogen bonds to form a one-dimensional chain along [010] direction. This structure crystallized in the monoclinic system with space group $P_{2_1/c}$.

The structure of 3,19-(2-bromobenzylidene)andrographolide, $C_{27}H_{33}BrO_5$, an andrographolide derivative, was semi-synthesized using andrographolide as a starting material. The structure contains three fused six-membered rings adopting chair conformations and a five-membered ring adopting an envelope conformation. The 2-bromophenyl group is twisted away from the attached ring. O—H---O hydrogen bonds in the structure form chains along the *b* axis which are interlinked *via* C—H---O interactions. This structure crystallized in the orthorhombic system with space group $P_{2_12_12_1}$.

The structure of 3,19-(2,6-dimethoxybenzylidene)andrographolide, $C_{29}H_{38}O_7$, an andrographolide analogue, was semi-synthesized from andrographolide. The structure contains three fused six-membered rings adopting chair conformations and five-membered ring adopting a twisted conformation. Intermolecular O—H---O hydrogen bonds link the structure into

chains along the *a* axis which are interlinked *via* C—H---π interactions. This structure crystallized in the orthorhombic system with the space group $P_{2_1}2_12_1$.

CHAPTER 1
INTRODUCTION

1.1 Preliminary

In this study, research on single crystal structure of natural products was carried out. This chapter describes basic X-ray crystallography theories and the characteristics of natural product.

1.2 X-ray Crystallography

X-ray Crystallography is a study of crystal structure through X-ray diffraction techniques. We can examine the internal structure of crystals through X-ray diffraction pattern which can be interpreted mathematically by certain computer software written to deal with diffraction pattern. This leads to an understanding of the molecular and crystal structure of a substance. Crystals are three dimensional ordered structures that can be described as repetition of identical unit cells. The unit cell is characterized by six parameters, with 3 axial lengths (a, b, c) and 3 interaxial angles (α, β, γ).

1.3 Generation of X-Ray

X-rays are produced by accelerating electrons towards a metallic target, which is maintained at high positive potential difference, V relative to the cathode. These high-speed electrons strike the metallic target and rapidly decelerate. If enough energy is obtained, it is able to eject an electron from the inner shell of the metal atom and the electrons from higher energy levels will fill up the vacancy. This electronic transition causes the generation of X-ray (Figure 1.1).

The relation of the energy to the frequency of the X-ray radiation is through the Planck's constant,

$$E = hv \qquad (1.1)$$

E = Radiation energy

h = Planck's constant

v = frequency of the X-ray radiation

$= \frac{c}{\lambda}$, c = speed of light

λ = wavelength.

$$\text{So, } E = \frac{hc}{\lambda} \qquad (1.2)$$

This indicates that the wavelength of radiation becomes smaller with larger energy transition.

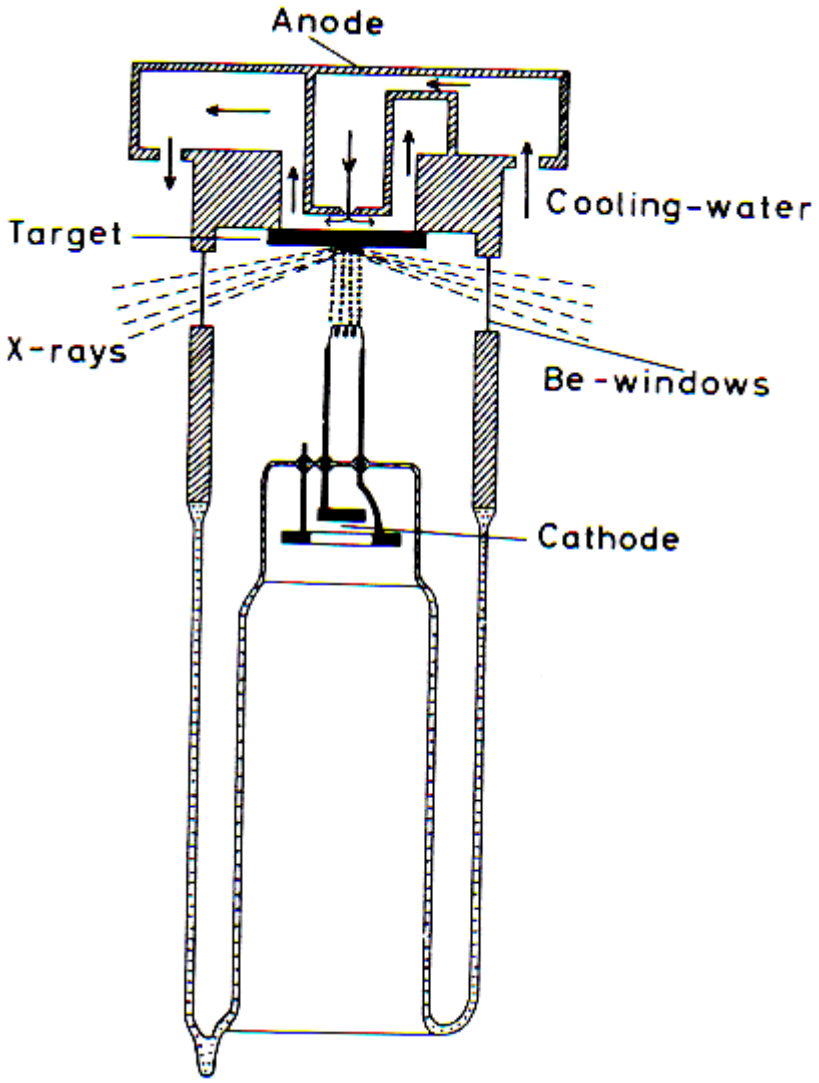

Figure 1.1 Schematic representation of X-ray tube (Luger, 1980).

1.4 X-ray Diffraction

Diffraction of an X-ray beam passing through a crystal occurs when the repeat distance in the crystal is about the same order of magnitude as the wavelength of the X-ray. The periodicity of crystal structures means that they can act as an X-ray diffraction grating. Crystals are used due to the diffraction pattern from one single molecule could be insignificant, but the many identical molecules in a crystal amplify the pattern.

X-ray beam generated from the X-ray tube contains not only the strong K_α line but also the weaker K_β line and the continuous spectrum. A selective filter has an atomic number 1 or 2 less than the target metal is chosen to absorb the K_β component, with a relatively much smaller loss of K_α (Stout & Jensen, 1989). In this single crystal X-ray structure determination research, a tunable graphite crystal monochromator is substituted for filter to select only the K_α line (λ = 0.71073 Å) emitted from the Mo X-ray source. The incident beam is then collimated by collimator system to produce a narrow beam (Bruker, 2005). The information about the lattice of a crystal is most easily obtained if the wavelength, λ is kept constant by using monochromatic X-ray. The monochromatic beam is then allowed to strike the crystal to be studied.

In 1912, Bragg noticed the similarity of diffraction to ordinary reflection (Stout & Jensen, 1989). By dealing the diffraction as reflection from plane in the lattice, he concluded a simple equation (Figure 1.2)

$$2d\sin\theta = n\lambda \qquad (1.3)$$

n = peak order

θ = angle between the incidence beam and the atomic planes

λ = wavelength of X-ray

d = interplanar spacing in the crystal lattice

This relation is known as Bragg's Law. For a known wavelength, λ and d spacing, the peak will occur at a particular θ according to the peak order, n.

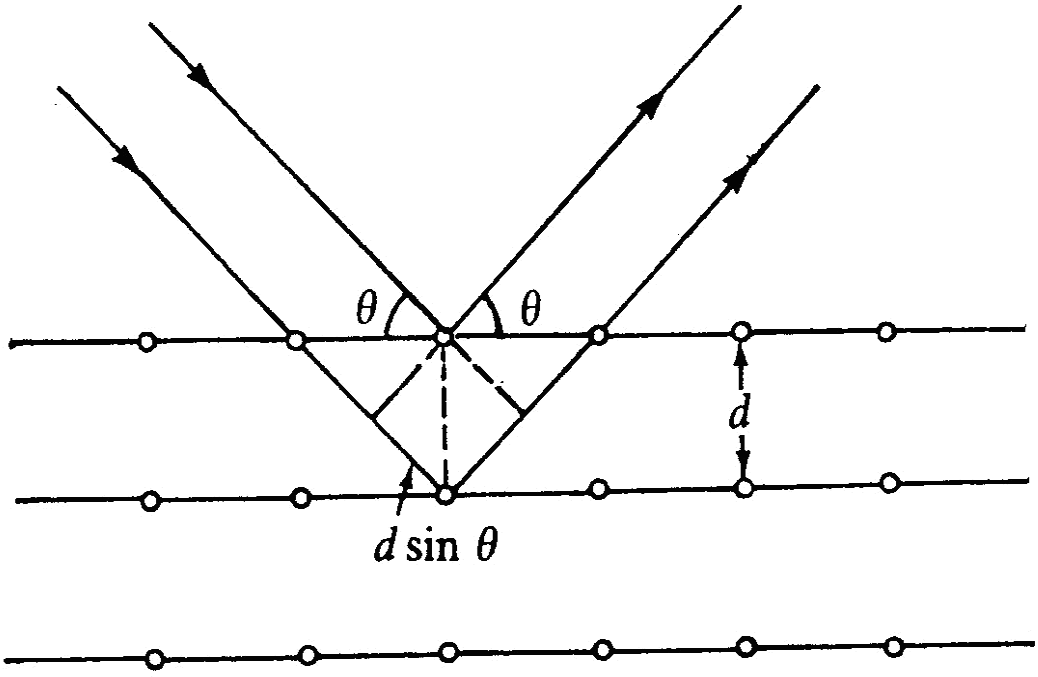

Figure 1.2 Bragg's Law (Halliday et al., 2001)

1.5 Reciprocal Lattice

From Bragg's law

$$\sin\theta = \frac{n\lambda}{2}\left(\frac{1}{d}\right) \qquad (1.4)$$

$\sin\theta$ is inversely proportional to d. Interpretation of X-ray diffraction patterns would be facilitated if the inverse relation between $\sin\theta$ and d could be replaced by a direct one by constructing a reciprocal lattice based on $\frac{1}{d}$, a quantity that varies directly as $\sin\theta$ (Stout & Jensen, 1989).

The relation between the crystal lattice and the reciprocal lattice may be expressed in terms of vectors (Glusker & Trueblood, 1985).

$$\vec{a^*} = \frac{\vec{b}\times\vec{c}}{\vec{a}\cdot\vec{b}\times\vec{c}} = \frac{\vec{b}\times\vec{c}}{V}$$

$$\vec{b^*} = \frac{\vec{c}\times\vec{a}}{\vec{a}\cdot\vec{b}\times\vec{c}} = \frac{\vec{c}\times\vec{a}}{V}$$

$$\vec{c^*} = \frac{\vec{a}\times\vec{b}}{\vec{a}\cdot\vec{b}\times\vec{c}} = \frac{\vec{a}\times\vec{b}}{V} \qquad (1.5)$$

$\vec{a}, \vec{b}, \vec{c}$ = unit vector in crystal lattice

$\vec{a^*}, \vec{b^*}, \vec{c^*}$ = unit vector in reciprocal lattice

For

$$V^* = \frac{1}{\vec{a} \cdot \vec{b} \times \vec{c}} = \frac{1}{V} \qquad (1.6)$$

where V = volume in crystal lattice

V^* = volume in reciprocal lattice

From equations (1.5) and (1.6), there is a translation between the vectors of the crystal lattice and the reciprocal lattice. $\vec{a^*}$, $\vec{b^*}$, $\vec{c^*}$ are perpendicular to the *bc, ac* and *ab* plane, respectively; likewise, $\vec{a^*}$ is perpendicular to both $\vec{b}$ and $\vec{c}$, $\vec{b}$ is perpendicular to both $\vec{a^*}$ and $\vec{c^*}$, and so on (Glusker & Trueblood, 1985).

1.6 Ewald Sphere

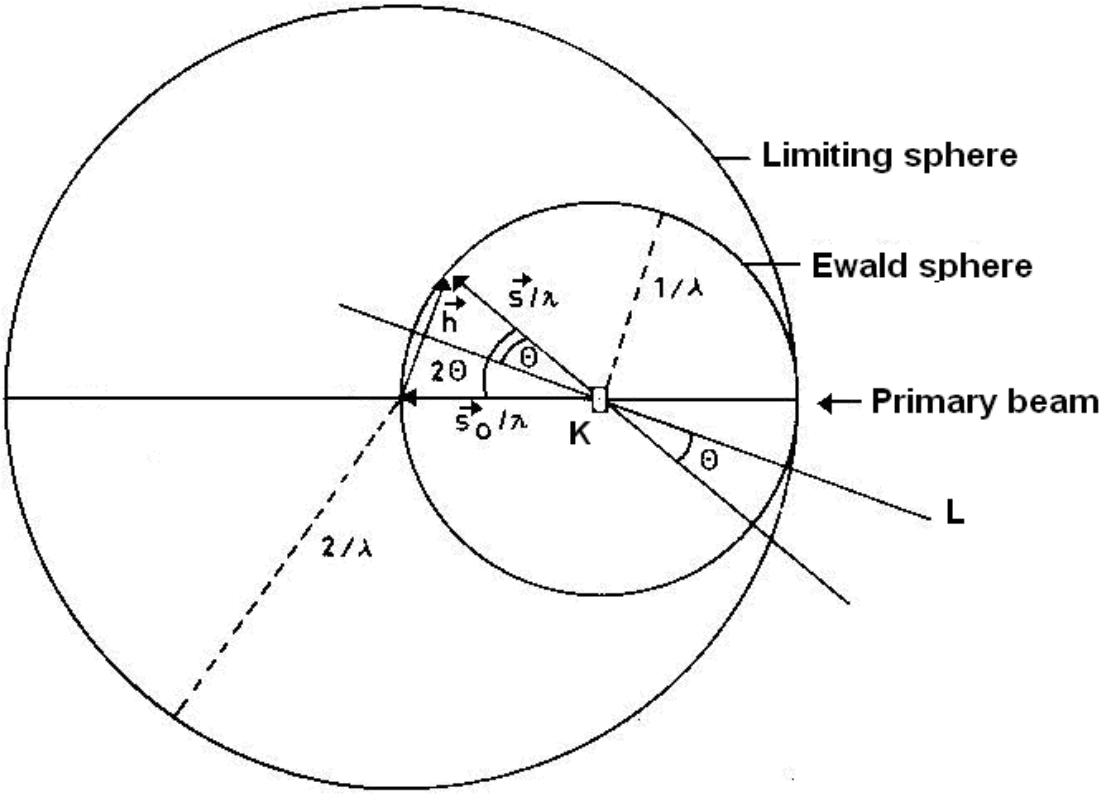

Figure 1.3 Ewald sphere (radius $\frac{1}{\lambda}$) and limiting sphere (radius $\frac{2}{\lambda}$) (Luger, 1980).

Paul Peter Ewald conceived Ewald's sphere, a sphere of radius $\frac{1}{\lambda}$, reciprocal lattice sphere. It is a geometric way that provides the condition for diffraction in reciprocal space. The sphere is centered on the crystal K and the origin of the reciprocal lattice lies in the transmitted beam, at the edge of the Ewald sphere. From the Ewald condition, consider a lattice plane L in a special position to cause diffraction (the diffraction position) where its normal vector h is on the surface of a sphere of radius $\frac{1}{\lambda}$ around K.

$$h = \frac{S - S_o}{\lambda} \tag{1.7}$$

where S_o is the unit vector in the direction of the primary beam, and S is the unit vector in the direction of the diffracted beam concerning a lattice plane with the normal vector h.

When the reciprocal of the normal vector's magnitude, d satisfies the Bragg's Law, diffraction of a lattice plane, L, happens (Figure 1.3).

$$\frac{|h|/2}{1/\lambda} = \sin\theta \qquad , d = \frac{1}{|h|} \tag{1.8}$$

$\therefore \qquad \lambda = 2d\sin\theta$

Since the diameter of the sphere is $\frac{2}{\lambda}$, each reciprocal lattice point within that distance of the origin can be brought into coincidence with its surface.

For a given radiation with fixed wavelength, λ the number of possible reflections is limited and only those reflections in reciprocal space inside the sphere of radius $\frac{2}{\lambda}$ can be observed. This sphere is called the “limiting sphere”. The limiting sphere has twice the radius of the Ewald sphere. Changing the wavelength of the incident radiation has the effect of enlarging (short λ) or shrinking (longer λ) the size of the sphere of reflection (Luger, 1980).

1.7 Natural Products

Natural products play an important role in the development of drugs since ancient times. Typically, when a natural product is found to be active, it is chemically modified to improve its properties as a result of advances made in synthesis and separation method as well as in biochemical techniques. In this research, the single crystal samples of natural products are obtained from the Department of Chemistry, Faculty of Sciences, Prince of Songkla University, Songkhla, Thailand, the Department of Biomedical Sciences, Faculty of Medicine and Health Science, Universiti Putra Malaysia, Serdang, Malaysia and the Laboratory of Natural Products, Institute of Bioscience, Universiti Putra Malaysia, Serdang, Malaysia.

The samples were analysed using Bruker SMART APEX II CCD area detector diffractometer in the X-ray Crystallography Unit, School of Physics, Universiti Sains Malaysia, Penang, Malaysia. X-ray Crystallography technique is an ideal method to determine and identify the structure of compounds and was used in this research on natural products.

Natural products are compounds produced by living systems such as plants, animals and microorganisms. They have played a major role in the development of organic chemistry. The major chemical and physical methods of structure elucidation have been developed during the study of natural products (Hanson, 2003).

Thus, there have been important advances in the study of compounds from natural sources to identify the structures of the compounds physically and chemically. The development of diffraction technique, such as X-ray crystallography has greatly simplified the structure elucidation of natural products.

Naturally existing compounds may be divided into three categories (Hanson, 2003). Firstly, there are compounds which occur in all cells and play a central role in the metabolism and reproduction of those cells. These compounds include nucleic acids and common amino acids and sugars. They are known as primary metabolites. Secondly, the high-molecular-weight polymerics like cellulose, lignin and protein which form the cellular structures. Finally, there are compounds that are characteristic of a limited range of

species. They are secondary metabolites. Drugs are only obtained from pure and best behaviour derivation of secondary metabolite natural products.

In late 18th century, scientists turned the traditional medicine into modern medicine study. The active constituents were isolated from plant, were structurally characterized and then synthesized in the laboratories. This led to the development of various instruments for structural analysis such as chromatography methods (paper chromatography, thin layer chromatography (TLC), column chromatography, high performance liquid chromatography (HPLC), gas-liquid chromatography, ion exchange chromatography etc.). These chromatography methods are used for the analysis or separation of mixture existing in natural products and other compounds.

X-ray crystallography is an ideal method to determine the limited and small amount sample. The lattice structure, chemical formula, bond lengths and bond angles can be determined more accurately by using X-ray crystallography method. Thus, the molecular and crystal structures of the compounds of natural products can also be precisely determined using this technique.

1.7.1 1-(2,4-Dihydroxy-6-methoxyphenyl)-3-(4-methoxyphenyl) propan-1-one

Rauwenhoffia siamensis Scheff. belongs to the family of Annonaceae, which is widely distributed in Thailand, Malaysia and Indonesia. *R. siamensis* has a local Thai name, Nom Maew, and has been used for biofragrance (Chulalaksananukul et al., 1998). The compound 1-(2,4-dihydroxy-6-methoxyphenyl)-3-(4-methoxyphenyl)propan-1-one was isolated from the leaves of *R. siamensis*, which were collected from Songkhla province in the southern part of Thailand. The naringin dihydrochalcone which is derivative of compound 1-(2,4-dihydroxy-6-methoxyphenyl)-3-(4-methoxyphenyl)propan-1-one was known as a sweetener (Shin et al., 1995).

1.7.2 1-3-Dihydroxy-2-methyl-9,10-anthraquinone

Prismatomeris malayana Ridl. or "Kradook Kai" in Thai is a medicinal plant. The extract from the root of this plant has been used as folk medicine for the treatment of skin diseases (Perry, 1980). 1-3-dihydroxy-2-methylanthra-9,10-anthraquinone, has been isolated from the roots of *Prismatomeris malayana* Ridl. which were collected from the Phuket province in the southern part of Thailand. Rubiadin was isolated before from *Rubia cordifolia* (Tripathi et al., 1997) and *Hedyotis capitellata* (Ahmad et al., 2005). It possesses an antioxidant property which is better than that of EDTA, Tris, manitol, vitamin E and *p*-benzoquinone (Tripathi et al., 1997).

1.7.3 12-(1,1-Dimethyl-2-propenyl)-5,9,10-trihydroxy-2,2-dimethyl-2*H*,6*H*-pyrano[3,2-*b*]xanthen-6-one

Compound 12-(1,1-dimethyl-2-propenyl)-5,9,10-trihydroxy-2,2-dimethyl-2*H*,6*H*-pyrano[3,2-*b*]xanthen-6-one, macluraxanthone, was isolated from the bark of *Cratoxylum formosum* ssp. *prunifolum*, a shrub which was collected from Nhongkhai province in the north-eastern part of Thailand. It is part of our continuing search for bioactive compounds obtained from Thai medicinal plants (Chantrapromma et al., 2004; Chantrapromma, Boonnak et al., 2005; Chantrapromma, Fun et al., 2005; Boonnak et al., 2005; Fun et al., 2005; Boonsri et al., 2005). Compound 12-(1,1-dimethyl-2-propenyl)-5,9,10-trihydroxy-2,2-dimethyl-2*H*,6*H*-pyrano[3,2-*b*]xanthen-6-one has been reported previously (Monache et al., 1981; Menache et al., 1983; Goh et al., 1992), but its X-ray crystal structure has not yet been reported.

1.7.4 3,19-(2-Bromobenzylidene)andrographolide

Andrographis paniculata Nees (Acanthaceae) is one of the most important medicinal plants, having been used in Chinese Traditional and Indian Ayurvedic medicine for a wide range of illnesses. Extensive research on this plant extract and its constituents has revealed various pharmacological properties including anticancer and immunostimulatory activities (Kumar et al., 2004).

The active chemical constituents reponsible for the pharmacological activities of *A. paniculata* are the labdane-type diterpene lactones, among which the major component is andrographolide. The stereochemistry of compound andrographolide has previously been established (Smith et al., 1982; Fujita et

al., 1984; Spek et al., 1987). Recent studies suggested that andrographolide is an interesting pharmacophore with anticancer and immunomodulatory activities and hence has the potential to be developed as a cancer chemotherapeutic agent (Stanslas et al., 2001; Rajagopal et al., 2003).

With the objective of developing andrographolide analogues with increased potency and good selectivity against human cancer cell lines, we subjected andrographolide to many semi-synthetic procedures yielding various structural analogues of this compound. Being one of the most promising anticancer andrographlide analogues, the compound of 3,19-(2-bromobenzyledine)andrographolide exhibited potency and better selectivity in NCI-USA cancer screening when compared with the parent compound of andrographolide. We have synthesized the compound of 3,19-(2-bromobenzyledine)andrographolide by reacting andrographolide with 2-bromobenzaldehyde at room temperature.

1.7.5 3,19-(2,6-Dimethoxybenzylidene)andrographolide

Andrographolide is a major component of labdane-type diterpene lactones isolated from *Andrographis paniculata* Nees. Previously, we have reported the crystal structure of 3,19-(2-bromobenzyledine)andrographolide (Ng et al., 2006), a lead antitumour agent of andrographolide analogues. In a subsequent study of derivatization of andrographolide, we have synthesized the compound of 3,19-(2,6-dimethoxybenzylidene)andrographolide, by reacting andrographolide with 2,6-dimethoxybenzaldehyde. These compounds were

synthesized with the aim of improving the antitumour potential of the parent compound andrographolide. Compound 3,19-(2,6-dimethoxybenzylidene)andrographolide was tested for cytotoxic activity in breast, lung and prostate cancer cell lines and it exhibited 50% inhibitory concentrations (IC50) in the submicromolar range. The X-ray crystal structure analysis of 3,19-(2,6-dimethoxybenzylidene)andrographolide was undertaken in order to establish its molecular structure and stereochemistry.

1.8 Objective

The main objective of this research is to determine the molecular and crystal structure of the natural products. Through the X-ray structural analysis we will be provided with the information of the bond lengths, bond angles, conformation, coordination and other crystallographic information of the molecular structure of different natural product compounds. From this information we will know how the molecules are linked and the kind of bonding that attached them together in the crystal structure. From the crystal structure packing, we can see how the molecules are arranged in different planes.

After the process of data collection and structure determination, the results of the structure analysis through X-ray crystallography methods will enable biologists, chemists and pharmacists to find the active compounds that are potential candidates for developing useful drug usage as well as to help them in their work towards searching and developing better medicine in the future.

1.9 Thesis Overview

In this thesis, the theory behind the X-ray structure analysis will be discussed in chapter 2. It is followed by explanations of the methods used in obtaining samples for analysis, data collection and data processing and structure determination. Chapter 4 will focus on the results of the data analysis and the interpretation of the structures determined. The conclusion of this study will be presented in chapter 5, which is the final chapter of the thesis. This final chapter will also include suggestions for future research.

CHAPTER 2
X-RAY STRUCTURE ANALYSIS

There are two important expressions in crystal structure determination. One of these is the structure factor **F**(*hkl*), which is the amplitude of reflection from the set of (*h,k,l*) planes. The second expression is the electron density, ρ(xyz) as a Fourier series involving the structure factors.

2.1 Argand Diagram

The reflection of *hkl* consists of combined scattering waves by all atoms in the structure. The waves are represented as vectors with real and imaginary components, by an Argand diagram (Figure 2.1).

From Figure 2.1,

$$\mathbf{f}_1 = f_1\cos\varphi_1 + \mathrm{i}f_1\sin\varphi_1 \qquad (2.1)$$

$$\mathbf{f}_2 = f_2\cos\varphi_2 + \mathrm{i}f_2\sin\varphi_2 \qquad (2.2)$$

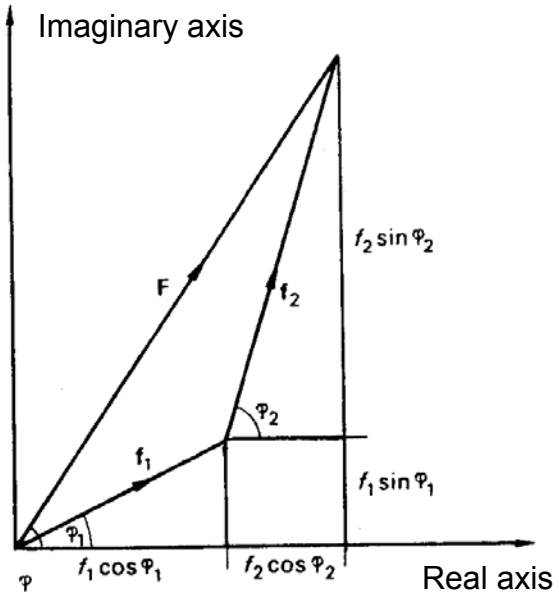

Figure 2.1 Combination of two waves as vectors, $f_1 e^{i\varphi_1}$ and $f_2 e^{i\varphi_2}$, on an Argand diagram (Ladd & Palmer, 1979).

De Moivre's theorem states that

$$e^{\pm i\varphi} = \cos\varphi \pm i\sin\varphi \tag{2.3}$$

from equation (2.1) and (2.2)

$$f_1 = f_1 e^{i\varphi_1} \qquad f_2 = f_2 e^{i\varphi_2} \tag{2.4}$$

Hence,

$$\mathbf{F} = f_1 e^{i\varphi_1} + f_2 e^{i\varphi_2} \tag{2.5}$$

2.2 Combination of N Waves

The combination of N waves, from (2.5)

$$\mathbf{F} = f_1 e^{i\varphi_1} + f_2 e^{i\varphi_2} + f_3 e^{i\varphi_3} + \ldots + f_j e^{i\varphi_j} + \ldots + f_N e^{i\varphi_N} \qquad (2.6)$$

or

$$\mathbf{F} = \sum_{j=1}^{N} f_j e^{i\varphi_j} \qquad (2.7)$$

In Figure 2.2, the equation 2.7 expresses a polygon of vectors. We can now derive **F** as

$$\mathbf{F} = |F| e^{i\varphi} \qquad (2.8)$$

The conjugate of **F** is $\mathbf{F}^*$

$$\mathbf{F}^* = |F| e^{-i\varphi} \qquad (2.9)$$

Hence, the amplitude |**F**| is given by

$$|F|^2 = \mathbf{FF}^* \qquad (2.10)$$

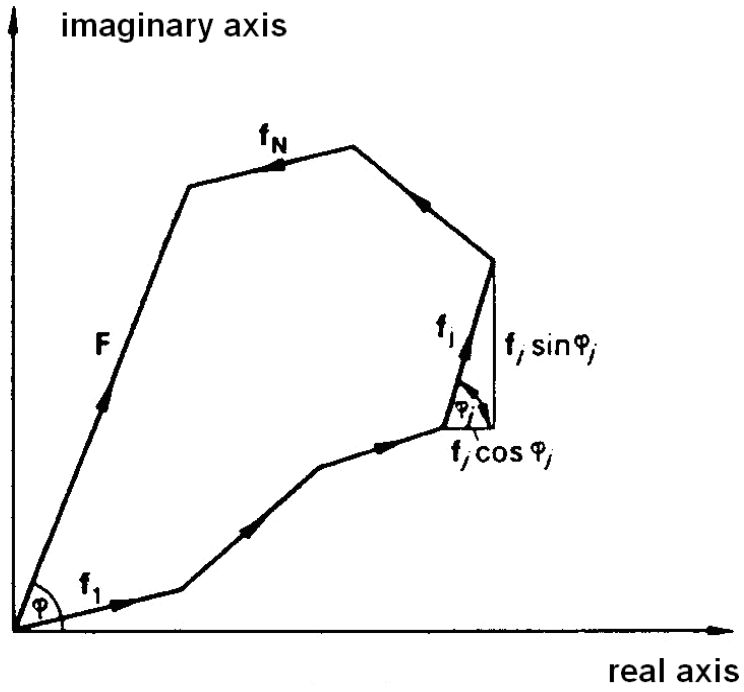

Figure 2.2 Combination of N waves (N=6) on an Argand digram; $\mathbf{F} = \sum_{j=1}^{6} f_j e^{i\varphi_j}$ (Ladd and Palmer, 1979).

2.3 Phase Difference

The expression of phase in terms of the positions of the atoms and the indices of the reflection is needed before the structure factor can be calculated. There is a phase differences of one cycle (2π radian or 360^o) between reflections from any set of (h,k,l) planes. The path difference associated with waves scattered by an atom j whose position relative to the origin is specified by the coordinates x_j, y_j, z_j is given as (Ladd and Palmer, 1979),

$$\delta_j = \lambda (hx_j + ky_j + lz_j) \qquad (2.11)$$

The corresponding phase difference (angular measure) is given by

$$\phi_j = (2\pi/\lambda)\,\delta_j$$

or
$$\phi_j = 2\pi\,(hx_j + ky_j + lz_j) \qquad (2.12)$$

2.4 The Atomic Scattering Factor

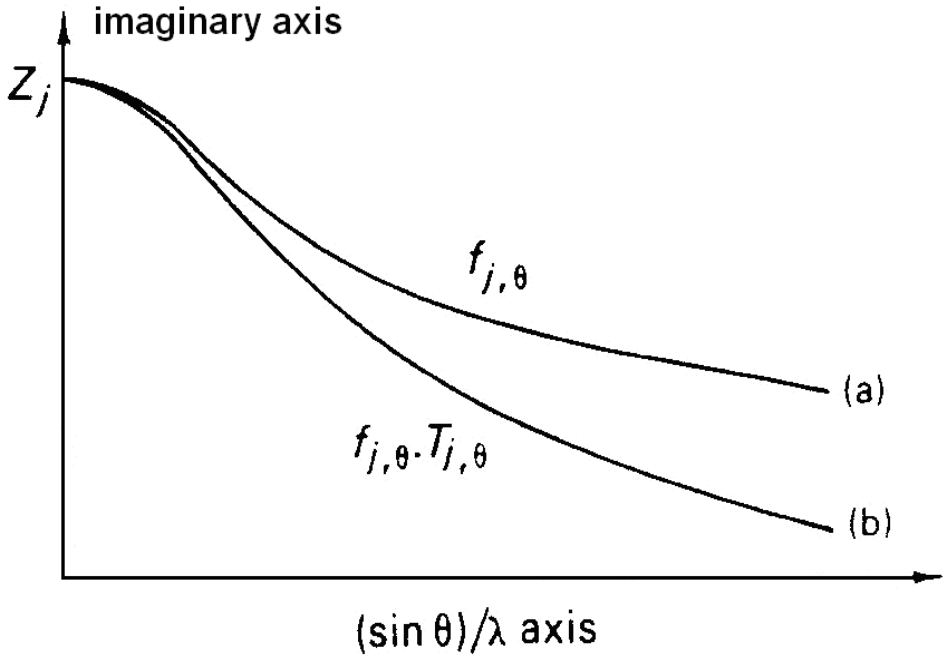

Figure 2.3 Atomic scattering factor:
(a) stationary atom, $f_{j,\theta}$
(b) atom corrected for thermal vibration, $f_{j,\theta}T_{j,\theta}$
(Ladd & Palmer, 1979).

The amplitudes of the waves scattered by atoms, the atomic scattering factors, f_j, is required to evaluate the combined scattering from the atoms in the unit cell. The atomic scattering factor depends upon the nature of the atom, the direction of scattering, the wavelength of X-rays used, and the thermal vibration of the atom (Ladd & Palmer, 1979). Initially, f_j is based upon the number of extranuclear of electrons in the atom. The atomic number of the *j*th atomic species, Z_j is its maximum value for a given atom *j*. f_j has its maximum value on the direction of the incident beam where $\sin\theta(hkl) = 0$.

$$f_{j,\theta(\theta=0)} = Z_j \tag{2.13}$$

f is measured in number of electrons.

Assumed isotropic vibration, where the temperature factor correction for atom *j* is

$$T_{j,\theta} = \exp[-B_j(\sin^2\theta)/\lambda^2] \tag{2.14}$$

B_j is the temperature factor of atom *j*, and is given as

$$B_j = 8\pi^2\overline{U_j^2} \tag{2.15}$$

$\overline{U_j^2}$, which is a function of temperature, is the mean square amplitude of vibration of atom *j* from its equilibrium position in a direction normal to the